Influence of Urease and Nitrification Inhibitors on Ammonium and Nitrate Supply and the Soil Microbial Population in Western Canadian Soils

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Abstract

Improving nitrogen use efficiency and limiting losses of N from the soil system is important both economically and environmentally. This study assessed the potential of using a urease inhibitor, Agrotain ®, or a nitrification inhibitor, nitrapyrin, to create a slow release effect similar to that found in sulfur coated urea in Western Canadian soils of the Brown and Black soil zones. The nitrapyrin significantly reduced the cumulative NO_3^- supply to the point of inhibition of nitrification for 21d in the Brown soil, but had little effect on the Black soil. Nitrapyrin had little effect on the supply of NH_4^+ in both soils. The Agrotain had some effect on NO_3^- supply and significantly reduced the NH_4^+ supply for 14d in the Brown soil. Differences between the two soils were consistent with results in other studies and attributed to pH and organic matter content differences. The total heterotrophic and *Nitrosomonas* microbial populations were enumerated using spread plates and most probable number assays. It was concluded that Agrotain had little effect on the microbial population, where as nitrapyrin reduced *Nitrosomonas* populations and increased total heterotrophic counts in both soils. Similarity between the results in this study and the literature suggest that the soils in Western Canada have similar responses to inhibitors as those characterized by the literature. Therefore, whether improved nitrogen use efficiency from the use of inhibitors in wheat in Western Canada would be large enough to justify the used of inhibitors will depend on whether local soil properties are conducive to promote volatilization, leaching and denitrification losses of N.

Introduction

Nitrogen dramatically improves yields and quality of crops when added to N deficient soils. In Western Canada, producers usually supply all of their N requirements at one time, either during the seeding operation or banded into the soil in late fall or early spring. This method can cause a large amount of available N to be released at one time, often not matching crop demand. Consequently, there may be a surplus of NO_3^- in the soil susceptible to immobilization, leaching and denitrification losses. This reduces fertilizer use efficiency and is a potential environmental threat.

When urea fertilizer or organic matter is broken down, the process of ammonification occurs:

$$R\text{-}NH_2 + H_2O \rightarrow NH_3 + R\text{—}OH + Energy$$

Potential for volatilization losses of NH_3 exist if urea is not in the soil or moisture is not available to rapidly react with NH_3 to form NH_4^+. As NH_4^+, N can be taken up by plants, held on the cation exchange or further oxidized to NO_3^- by microorganisims in a process called nitrification. Nitrification occurs when *Nitrosomonas* oxidizes NH_4^+ to NO_2^- which is further oxidized to NO_3^- by *Nitrobacter*. Excess NO_3^- is undesirable in the soil system because it is susceptible to leaching and denitrification, becoming lost from the system.

In order to reduce losses due to excess NO_3^- from large, one time applications of N, split applications may be performed to better match supply and crop demand. However, split applications require additional passes through the field and post emergent applications are not always as effective as pre-emergent applications, relying on favourable weather conditions after application (Raun and Johnson, 1999). Products have been developed to decreases losses and increase nitrogen use efficiency. Most commonly, N is coated to reduce solubility and slow down the microbial and enzymatic processes that transform N (Havlin *et al*., 1999).

Sulfur coated urea (SCU) has been shown to improve N use efficiency, however, these fertilizer products are usually cost prohibitive on a large scale (Singh and Singh, 1989). Two products are commercially available to inhibit microbial and enzymatic transformations of N to plant available forms. Nitrapyrin (2-chloro-6-trichloromethyl-pyridine), marketed by Dow Agro-Sciences as N-Serve® (available only in the United States), inhibits a complex of membrane-bound proteins, ammonia monooxygenase (AMO) in *Nitrosomonas*, preventing the conversion of NH_4^+ to NO_2^- (McCarty, 1999) . Nitrapyrin is also proposed to bind to copper making it unavailable to activate enzymes required in NH_4^+ oxidation (Wolt, 2000). Inhibition of nitrification reduces losses of N by preventing excess amounts of NO_3^- that can easily be lost through leaching or denitrification (Walters and Malzer, 1990). N-(n-Butyl)-thiophosphoric triamide (NBPT), produced by Agrotain International, inhibits the process of ammonification. In the soil NBPT converts to an oxon analog that occupies the urease active site, inactivating the enzyme (Creason *et* al., 1990). Preventing ammonification prevents volatilization of NH_3 if urea is broadcast and reduces the supply of NH_4^+ available for nitrification, thereby reducing the amount of NO_3^- present (Havlin *et al*., 1999).

The performance of these inhibitors has been variable. Cochran *et al*. (1973) and Strong, *et al*. (1992) found little yield response to N-Serve in trials with wheat on irrigated and dryland soils in the United States and Australia respectively. However, Gasser (1965) found increased N recovery and yield. In Alberta, it was concluded that losses from N fertilizer during the cropping season in spring season crops are minimal and only fall seeded winter cereals with increased potential for N loss may experience some benefit from low levels of nitrification inhibitors (Malhi and Nyborg, 1988). Research on Agrotain® has found reduced N losses and increased yields in corn in the United States (Hendrickson and Douglass, 1993). In Manitoba, significant yield and protein increases and improved N use efficiency was dependent on environmental conditions (Grant, 2004, Rawluk *et al*., 2000). Overall, few studies have been conducted in Canada, especially in the Prairie Provinces to confirm increased N use efficiency.

The purpose of this study is to see if treatments with urea fertilizer and the inhibitors provide similar release curves to SCU in loamy soils from Western Canada. If this is shown, it is possible that inhibitors could provide a more economical solution to improving N fertilizer use

efficiency than SCU. More specifically the objectives are to 1) determine effect of inhibitors on supply rates of NO_3^- and NH_4^+ over time and 2) evaluate the effect of the inhibitors on total heterotrophic and nitrifying microbial populations.

Materials and Method

Two soils were collected from the surface (0-15cm) by hand with a shovel (about 4L bulk). The Black soil, a Black Chernozem from the Blaine Lake association was collected August 25, 2002 near Jackfish Lake, SK (SE 28-48-28 W3). The Brown soil, a Brown Chernozem of the Milk River association was collected August 31, 2002 near Foremost, AB (NE 8-7-11 W4). Samples were air-dried, uniformly mixed and sieved through a 2mm sieve. Sub-samples were sent to Enviro-Test Labs (Saskatoon, SK) to be tested for NO_3^- N, organic matter and pH (Table 1). NBPT was obtained as Agrotain® from Agricore United in North Battleford, SK, and nitrapyrin was obtained from SIGMA Labs (St. Louis, MO).

Table 1. Selected properties of soils used (0-6 inch depth).

Soil	Texture	pH 1S:2W	Organic Matter (%)	NO_3^- -N (lb/ac)
Moist Black	Clay loam	5.5	5.6	71
Brown	Clay loam	7.3	2.7	>72

To determine the supply rate of NO_3^- and NH_4^+ dried, sieved soil was measured into 40-dram vial lids flush to the surface (11g and 10g per lid for the Black and Brown soil respectively) and 5mL of each treatment was added to each lid to bring the soil up to 80% of field capacity. Two lids were then sandwiched around a resin membrane and wrapped in parafilm to reduce evaporation.

Five different treatments were set up and replicated three times; 1) untreated, 2) urea (46-0-0), 3) urea + nitrapyrin, 4) urea + Agrotain ®, and 5) SCU. Treatment 1 had distilled water added to it with no other amendments. For treatments 2, 3 and 4 urea was dissolved in distilled water (to ensure even dispersal in the soil) to supply 100 µg N per g of soil in 5mL of solution. In treatment 2 the urea solution was simply added to the soil. For treatment 3 liquid Agrotain® was added to the urea solution at the maximum label rate and was then applied to the soil. Treatment 4 had nitrapyrin applied the recommended rate for N-Serve. As nitrapyrin is not soluble in water, the active ingredient was dissolved in methanol (Lewis and Stefanson, 1973), the methanol solution was added to the total amount of Black and Brown soil required for all three replicates, mixed well and weighed into the lids. Finally, 5mL of the urea solution was added. For treatment 5 small prills of SCU were selected by weight to supply the equivalent amount of N supplied by urea. Prills were placed equal distances throughout the soil and 5mL of distilled water was added per lid.

The cation and anion exchange membranes were obtained from Western Ag Innovations Inc. (Saskatoon, SK). The resin membranes were cut into 5.5 x 5.5 cm squares, and soaked in ionic solutions of lower selectivity than NO_3^- and NH_4^+ to saturate the membranes with an ion that

could be easily replaced (Qian and Schoenau, 2002). Cation membranes were soaked in 0.5M HCl for 24 hrs and anion membranes were soaked in 1M $NaHCO_3$ solution that was changed three times in 24 hrs. Before being used in the soils the membranes were rinsed in distilled water. Treatments were incubated at 25°C and the membranes were replaced at 1, 3, 7,14,21,28 and 35 days after treatment. When membranes were removed, soil was rinsed off in a stream of distilled water and they were placed in 40-dram vials (both the anion and cation for each treatment) with 50mL of 0.5M HCl and placed on a shaker for 1 hr at 25°C to desorb the NO_3^- and NH_4^+ ions (Qian and Schoenau, 2000). Solutions were analyzed by colorimetry with a Technicon Autoanalyzer (Technicon Industrial Systems) to detect the amount of NO_3^- and NH_4^+ in solution. After being analyzed supply rates of μg NO_3^- and NH_4^+ sorbed per $10cm^2$ were calculated. At the conclusion of the experiment, 10g of each replicate was extracted with 50mL 2M KCl by shaking for 1 h at 25°C and then filtered through VWR 454 filter paper into vials to be analyzed with Technicon Autoanalyzer (Technicon Industrial Systems) for any remaining NO_3^- and NH_4^+ (Bremner and Keeney, 1966).

To evaluate the effects of the treatments on the microbial population both the total heterotrophic and nitrifying populations were enumerated. Before the treatments were applied, soil dilutions were spread plated on 1/10 Bacto Tryptic Soy Agar (Difco. Sparks, MD) and incubated at 27°C for two days. Plates containing between 25-250 colony forming units were counted, the same procedure was completed on each of the treatments at the completion of the experiment.

To enumerate the nitrifying bacteria a most probable number technique (MPN) was used because nitrifiers are difficult to isolate due to slow growth and susceptibility to contamination (Schmidt and Belser, 1994). When preparing treatments for the membranes, 100g of soil was put in 60-dram vials and treated with the appropriate amount of each treatment. Treatments were kept at 80% moisture and each time the membranes were changed 10g was used to prepare serial dilutions of 10^{-5} in phosphate buffer solution and 0.1mL used to inoculate 1mL of ammonium-calcium carbonate medium. The MPN plates were incubated at 25°C for six weeks when Nit 1 and Nit 2 (reagents pre-made by Biomerieux, Montreal QP) were added to each well. A reddish colour development indicated positive test for *Nitrosomonas*. If results were negative a pinch of zinc dust was added to see if the NO_2^- produced was further oxidized to NO_3^- by *Nitrobacter.* If the oxidation occurred, the zinc dust would allow a reddish colour development. The most probable numbers were estimated between the highest dilution with a positive result and the first negative result. Due to the small number of replicates, only estimates could be made.

Results and Discussion

An analysis of variance (ANOVA) was calculated between treatments on each day individually to determine if differences were significant at the 95% level. The number of days the supply rate of NH_4^+ and NO_3^- was measured in the Brown soil was reduced to 21d during the experiment due to an apparent increasing lack of contact between the membrane and the soil. Changing membranes always resulted in a loss of a small amount of soil; however, it became significant when membrane contact became degraded. Also, due to the small amount of soil it was difficult to utilize SCU properly. Small prills were selected to meet urea rates, however, selecting small prills maybe selecting for urea with thinner coating, causing a high release of urea. Compounding this problem, the SCU was placed in a concentrated spot in the soil, and could

easily develop 'hot spots' on the membranes resulting in higher levels of supply than would be expected in a field situation. This was reflected in the very high supply rate of SCU compared to all treatments and for these reasons it is believed that the SCU treatment results may be of limited validity.

The effect of nitrapyrin in the soil resulted in significant decreases in the NO_3^- supply in the Brown soil 7, 14 and 21d after treatment (Figure 1). Reduction was noted in the Black soil but was not statistically significant. The application of Agrotain® also resulted in a decrease in NO_3^- supply on day 1, 3 and 7 that was significant in the Brown soil. The higher NO_3^- supply from the Agrotain® than nitrapyrin suggests that Agrotain® may have reduced the NO_3^- supplied from the urea but had no effect on the release from mineralization, where the nitrapyrin inhibited mineralization from organic matter as well.

The effect of nitrapyrin on the supply of NH_4^+ was not significantly different from the supply from the urea treatment, as expected due to the mode of action of nitrapyrin (Figure 1). Agrotain® was found to significantly decrease NH_4^+ supply in the Brown soil on 1, 3 and 7d after treatment (Figure 1). On day 14 there was still a reduced supply compared to the urea alone but it was not significant at the 95% level. This is consistent with the 14d control window stated on the label for the rate applied. A similar effect was noted in the Black soil, but differences were only significant for 1 and 3 d after treatment, with an insignificant reduction of NH_4^+ supply on day 7.

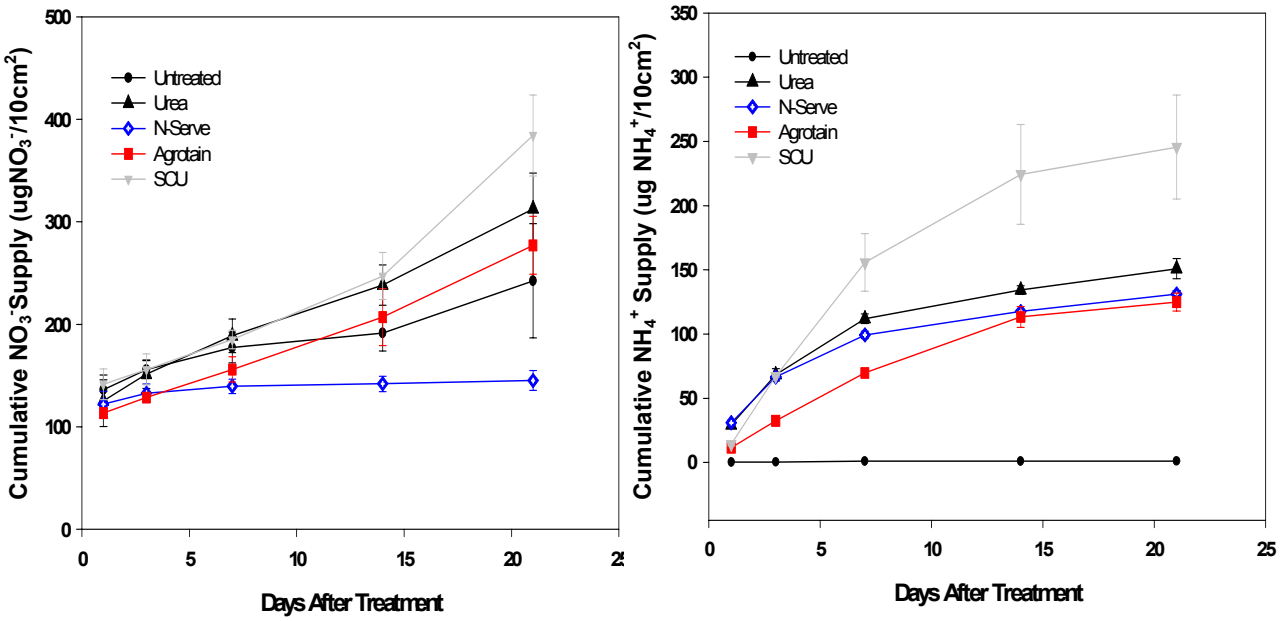

Figure 1. Cumulative NO_3^- (right graph) and NH_4^+ (left graph) supply rate in the Brown soil.

Decreases were noted in the nitrifying population in both soils when treated with nitrapyrin, but not until later in the experiment, possibly indicating a delay in response to the application of nitrapyrin (Figure 2). Nitrapyrin was also found to cause an increase in the total heterotrophic

population in the both soils (Figure 3). No effect on the population of nitrifiers or on the total heterotrophic population in the soil was noted due to the application of Agrotain®.

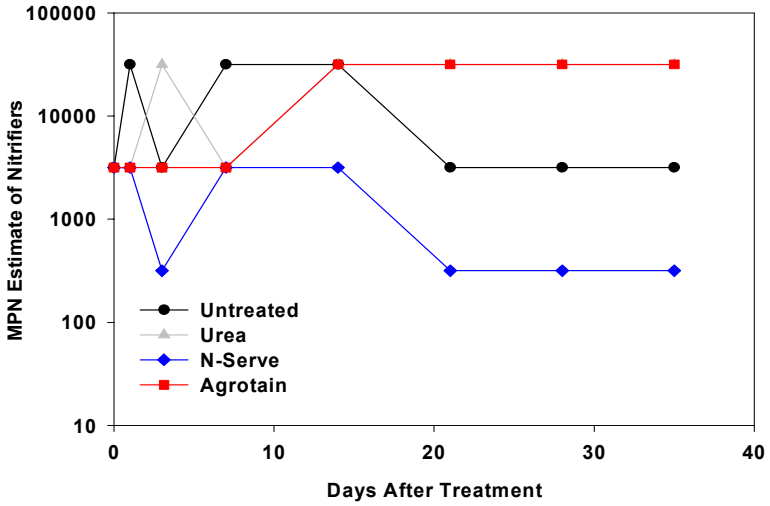

Figure 2. Most probable number estimate of nitrifiers for the Black soil according to treatment.

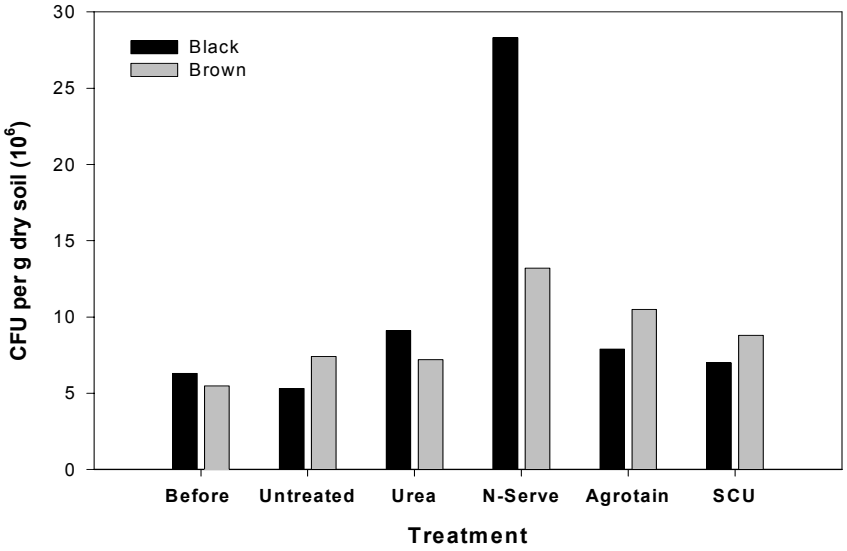

Figure 3. Colony forming units per g dry soil of hetertrophic microorganisms.

Differences in the response of the two soils to nitrapyrin are most likely due to the organic matter differences. Adsorption of nitrapyrin is positively correlated with soil organic carbon and decreased activity results because the nitrapyrin is unable to chelate copper or react with AMO (Wolt, 2000). As the Black soil had a considerably higher organic matter content (5.6%) but the same texture compared to the Brown soil (2.7% organic matter), it is reasonable to assume some of the reduced activity may be attributed to the organic matter in the Black soil. The Black soil did show a considerable reduction in the population of nitrifying bacteria when treated with

nitrapyin, similar to the Brown soil. Therefore, the lack of inhibition may be due to the dual mechanism of inhibition (affecting the chelation of copper as well as the microbes). The inhibitor may have been less effective because adsorption reduced the interaction with copper, rather than reducing the effect on the microbial population. Additionally, the soil pH may have had a small effect, as half lives of nitrapyrin have been determined to be reduced in acidic (<6.4) or basic (>7.2) pH, however the hydrolysis of the nitrapyrin is independent of pH at a range from 3.2 to 8.4 (Wolt, 2000). Therefore pH may have contributed slightly to the performance of nitrapyrin as the Black soil was slightly acidic (pH 5.5), but it is unlikely to have had a large effect.

Similar to the response of the soils to nitrapyrin, the Brown soil showed more response than the Black soil to the application of Agrotain®. Once again, organic matter differences could contribute to this difference as Carmona *et al*. (1990) found that increasing organic matter and straw residue decreased the effectiveness of Agrotain. Additionally, the persistence of Agrotain® decreases at low pH compared to neutral and alkaline soils (Hendrickson and Douglass, 1993). Therefore, the combination of high organic matter and low pH could result in the weaker and shorter response noted in the Black soil.

It is expected that results would show slightly higher supply rates than what would occur in the soil in the field due to lower temperature and drier soil moisture status in the field. Both of the inhibitors are more effective at lower temperatures, as there is less enzyme activity to inhibit and the degradation of the inhibitors proceeds more slowly (Clay *et al.*, 1990). However, within limitations it may be suggested that the response to Agrotain® and nitrapyrin applications that was found in this study were generally consistent with results found in the literature regarding supplies of NO_3^- and NH_4^+. The contradictory results of this study and the literature on the SCU can be attributed to the small sample size and the inability to effectively utilize granules of SCU.

Conclusion:

Although the results from SCU were not available to make comparisons on release rates between SCU and the inhibitors, the similarity in the release rates of NH_4^+ and NO_3^- in this study and the literature support the results. It is reasonable to conclude that the soils in Western Canada would have similar release rates as those characterized in the literature. Therefore, as past studies show, the apparent largest factor on whether yield and protein increases in wheat in Western Canada would be large enough to make applications of inhibitors economically feasible will depend largely on the potential for loss of N. Environmental conditions and local soil properties must be conducive to promote volatilization, leaching and denitrification losses of N. If these conditions do not exist the slowed release of available N from the fertilizer applied with inhibitors does not appear to have significant yield response or improved nitrogen use efficiency to justify the costs. Further study involving both plants and plant root simulator probes (PRS®) in the field would be a beneficial extension. Supply rates could be evaluated using both plant and probe concentrations and the yield and protein response in the crop could be evaluated to better determine the effects of inhibitors.

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